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SANDEELS IN THE DIETS OF SEALS: APPLICATION OF NOVEL AND CONVENTIONAL METHODS OF ANALYSIS TO FAECES FROM SEALS IN THE MORAY FIRTH AREA OF SCOTLAND

G.J. PIERCE, P.R. BOYLE, J.S.W. DIACK* AND I. CLARK

Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen, AB9 2TN
*Present address: Department of Medical Genetics, Medical School, Aberdeen Royal Infirmary, Aberdeen

Serological methods for prey identification have been applied to detection of residues of sandeel (Ammodytidae) protein in faeces of common seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) from the Moray Firth, north-east Scotland. Antisera raised to muscle protein from *Ammodytes marinus* were evaluated by testing their reactions with protein extracts made from a range of North Sea fish species and protein residues in *in vitro* digestates, seal digestive tracts and seal faeces. It was concluded that, using fused rocket immuno-electrophoresis, linkage of precipitin peaks from unknown samples with peaks from standard sandeel extract was a reliable indicator of the presence of sandeel in the unknown sample. Seasonal variation in the incidence of sandeels in common seal diet in the Moray Firth was examined by identifying otoliths, bones, and proteins, and all three methods indicated that sandeels occurred in the majority of samples tested in the summer, but were less important during the winter. Proteins were detected in fewer samples than otoliths, particularly in February and March. Possible reasons for this difference are discussed. Serological identification of sandeel proteins is potentially applicable to dietary studies on all marine predators.

INTRODUCTION

Serological methods of prey identification are well-established in studies of marine trophic interactions (*e.g.* Boreham & Ohiagu, 1978; Boyle *et al.*, 1986; Feller & Gallagher, 1982; Feller *et al.*, 1979; Grisley & Boyle, 1985, 1988) and have recently been applied to the diets of seabirds (Walter *et al.*, 1986) and marine mammals (Pierce *et al.*, 1990b).

Antisera raised to muscle protein extracts from salmon (*Salmo salar* Linnaeus) reacted specifically with Salmonidae proteins in digestive tract contents of common seals (*Phoca vitulina* Linnaeus), grey seals (*Halichoerus grypus* Fabricius), and bottle-nosed dolphins (*Tursiops truncatus* Montagu), and in faeces from seals fed on salmon (Pierce *et al.*, 1990b). However, the serological approach has not previously been applied to screening seal faeces collected in the wild.

The selection of sandeels for a field test of serological identification of prey in seal faeces is appropriate for two reasons. Firstly, sandeels (Ammodytidae) are an important food source for both grey and common seals (McConnell *et al.*, 1984; Pierce *et al.*, 1989, 1990a, in press a, b), and sandeel protein residues are likely to be present in a significant proportion of field samples. Secondly, sandeel bones and otoliths are frequently found

intact in seal faeces (*e.g.* McConnell *et al.*, 1984; Pierce *et al.*, 1990a, in press b), allowing independent verification of the presence of sandeel remains.

In the wider ecological context, sandeels are important prey for a variety of other marine predators: fish (Daan, 1989; ICES, 1989), seabirds (Bailey, 1986), and cetaceans (*e.g.* Payne *et al.*, 1986), and are also fished commercially in Shetland and other parts of the North Sea (Kunzlik, 1989).

Although sandeel hard parts are readily identifiable in seal faeces, there are various reasons why hard parts might be absent from these and other types of sample, *e.g.* protein residues and hard parts may pass through predator digestive tracts at different rates, hard parts may be fragmented (as by a gizzard), or, in the case of stomach lavages, might not be collected in the sample. In such circumstances, and for prey with friable bones (or no bones), serological methods may represent the only possible means of prey identification.

The Moray Firth, Scotland, has resident populations of both common seals and grey seals: at least 1000 common seals live in the Beauly, Cromarty, Dornoch and Inverness Firths (P. Thompson, unpublished data), and over 300 grey seals were counted in the Dornoch Firth in the summer of 1987. Caves and beaches at Helmsdale, to the north of the Moray Firth, are used by breeding grey seals (DAFS, unpublished data).

The present paper describes tests of new antisera raised to *Ammodytes marinus* (Raitt), and the application of these antisera to identification of sandeel proteins in faeces of common seals and grey seals, collected in the Moray Firth area during 1987 and 1988. The incidence of sandeels evaluated using the serological approach is compared with results from sandeel bones and otoliths.

METHODS

Methods for sample preparation, protein extraction, raising and testing antisera, and screening samples, are as described in Pierce *et al.* (1990b) unless otherwise stated.

Fish protein extracts

Muscle protein extracts (50 mg protein ml^{-1}) were prepared from *Ammodytes marinus*, for antiserum production, and from a range of North Sea fish species for testing antiserum specificity (see Table 1). Further extracts were made from cod, herring, mackerel, salmon, sandeel, and whiting, digested *in vitro* for 30 min, also from sandeel digested *in vitro* for 10, 20, 60, 90 and 120 min.

Seal digestive tracts and faeces

Protein extracts were made from digestive tract contents of four grey seals: three guts contained large numbers of sandeel otoliths and bones; the other, remains of lumpsuckers (*Cyclopterus lumpus* Linnaeus), clupeids and gadids, but no sandeels. Protein extracts were prepared from faeces of captive common and grey seals which had been fed on (a) salmon, (b) cod, (c) mackerel, and (d) herring and sprat.

Table 1. *Species of fish used for testing antiserum specificity. Taxonomic authorities for all species are given in Wheeler (1969)*

		(a) Selachii	
Pleurotremata:	Scyliorhinidae:	Dogfish	*Scyliorhinus* sp.
	Squaloidae:	Spur-dog	*Squalus acanthias*
Hypertremata:	Rajiidae:	Starry ray	*Raja radiata*
		(b) Pisces	
Isospondyli:	Argentinidae:	Argentine	*Argentina sphyraena*
	Clupeidae:	Herring	*Clupea harengus*
	Salmonidae:	Salmon	*Salmo salar*
		Trout	*Salmo trutta*
Anacanthini:	Gadidae	Cod	*Gadus morhua*
		4-bearded rockling	*Rhinonemus cimbrius*
		Hake	*Merluccius merluccius*
		Norway pout	*Trisopterus esmarkii*
		Pollack	*Pollachius pollachius*
		Poor-cod	*Trisopterus minutus*
		Silvery pout	*Gadiculus argenteus*
		Whiting	*Merlangius merlangus*
Percomorphi:	Carangidae:	Scad	*Trachurus trachurus*
	Scombridae:	Mackerel	*Scomber scombrus*
	Callionymidae:	Dragonet	*Callionymus lyra*
Scleroparei:	Scorpaenidae:	Norway haddock	*Sebastes viviparus*
	Triglidae	Red gurnard	*Aspitrigla cuculus*
	Cottidae:	Bullrout	*Myoxocephalus scorpius*
Heterosomata:	Bothidae:	Megrim	*Lepidorhombus whiffiagonis*
	Pleuronectidae:	Flounder	*Platichthys flesus*
		Long rough dab	*Hippoglossoides platessoides*
		Plaice	*Pleuronectes platessa*
		Witch	*Glyptocephalus cynoglossus*
	Soleidae:	Dover sole	*Solea solea*

Throughout 1988, regular visits were made to common seal haul-out sites, principally intertidal sandbanks, in the Beauly, Cromarty, Dornoch and Inverness Firths (Figure 1). All faeces found were collected. Common seal faeces were obtained in all months but grey seal faeces were found only in the months April to July. Grey seal faeces were also collected at Helmsdale in April. Protein extracts were made from all samples, as above. For months in which more than 20 samples of either species were obtained, 20 of those samples were randomly selected for testing.

Raising antisera

Two Dutch rabbits (K, L) were immunized with sandeel muscle protein extract. The protocol was similar to that described in Pierce *et al.* (1990b), except that booster injections were given 1 month (0·5 ml extract + 0·5 ml Freunds incomplete adjuvant) and 2 months (1 ml extract) after the initial injection.

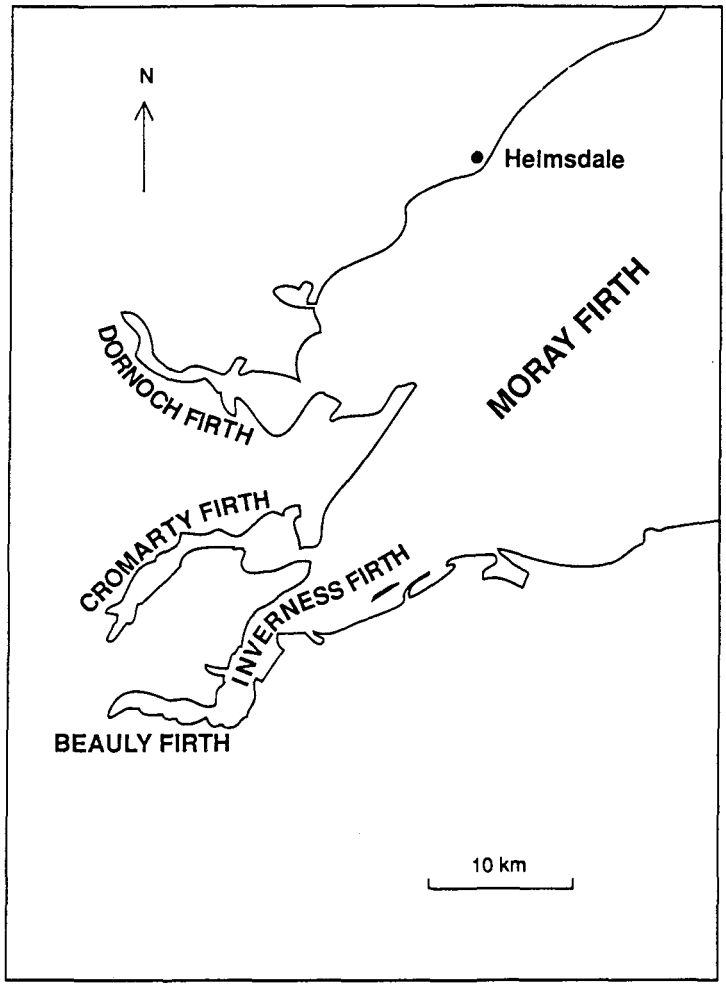

Figure 1. The Moray Firth study area.

Testing antisera

Antiserum titre was evaluated subjectively using homologous crossed immuno-electrophoresis (CIE; Weeke, 1973).

Reactions of other proteins with the antisera were evaluated using fused rocket immuno-electrophoresis (FRIE; Svendsen, 1973). Reactions were assessed visually, noting the number of precipitin peaks arising from the unknown samples, and the number of links between peaks from the unknowns and peaks from the adjacent standards. Linking, in which adjacent peaks form a continuous smooth curve, indicates that the proteins were indistinguishable to the antiserum. Where a peak terminated on contact with an adjacent peak, but the two peaks met at a more or less acute angle, the reaction was scored as a 'partial link'.

To test reactions with the chronological series of sandeel *in vitro* digestates (50 mg ml^{-1}), raw sandeel protein extract (10 mg ml^{-1}) was used as the standard. For subsequent tests, two sets of standards were used: raw sandeel protein (Standard 1) and sandeel digestate (30 min; Standard 2). Samples were arranged so that each unknown was adjacent to both standards. This procedure was used for:
(a) Raw protein from other fish species (10 mg ml^{-1});
(b) *In vitro* digestates from other fish species (50 mg ml^{-1});
(c) Digestive tract contents (50 mg ml^{-1});
(d) Faeces from captive seals (50 mg ml^{-1});
(e) Faeces from grey seals at Helmsdale, of which 19 out of 21 contained hard remains of sandeels (50 mg ml^{-1}).

Evaluation of the incidence of sandeels in seal faeces

Proteins

Extracts from each wild faecal sample were run into antiserum K, with sandeel *in vitro* digestate (30 min) used as the standard. Reactions were scored as negative (no peaks), positive (peaks but no linkage) and linked (full linkage to peaks in the standards). The number of linked peaks was counted in each case. Each author scored all gels independently, with gels being repeated where there was any doubt as to the presence of links.

Hard remains

Sandeel otoliths were counted and sandeel bones were scored as present or absent. Otoliths of different species of sandeel could not be distinguished: although, for example, otoliths of the greater sandeel (*Hyperoplus lanceolatus* Lesauvage) are often larger than those of *Ammodytes marinus* or *A. tobianus* (Linnaeus), the size ranges are overlapping and all species have very similar otoliths (Harkonen, 1986). Other prey remains were also identified and other otoliths were counted. The co-occurrence of hard remains and proteins was evaluated using Mann-Whitney U tests, Spearman's rank correlation coefficients, and analysis of variance. Otolith numbers were transformed to logarithms ($\log_{10}[N+1]$) prior to analysis of variance.

RESULTS

Antiserum testing

Reactions with sandeel protein

Both antisera showed reasonably strong reactions with proteins in raw sandeel protein extracts (Figure 2). Antiserum L was of higher titre than antiserum K (variability between rabbits is commonly seen when producing antisera). The antisera reacted with *in vitro* digested sandeel protein, and linkage was seen between raw and *in vitro* digested proteins (Figure 3).

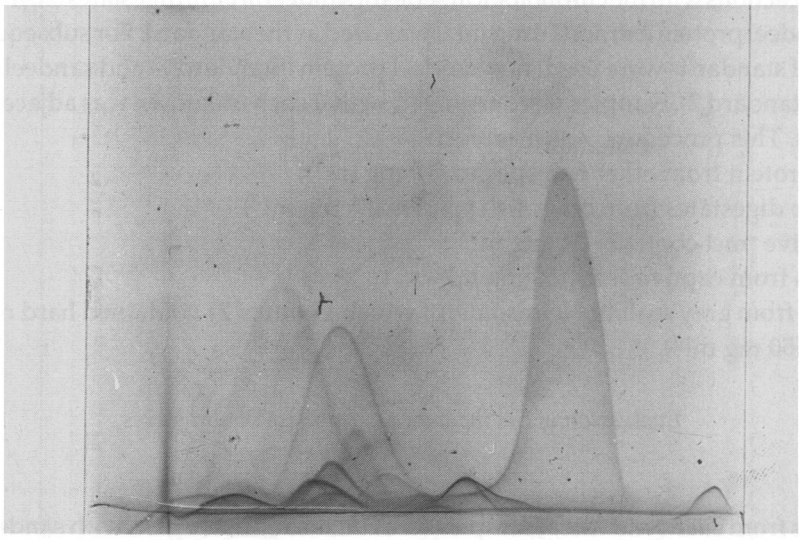

Figure 2. Reaction between sandeel antiserum L and homologous muscle protein extracts, visualised by CIE (rabbit L). Each peak represents an antigen in the sandeel extract which is recognised by the antiserum.

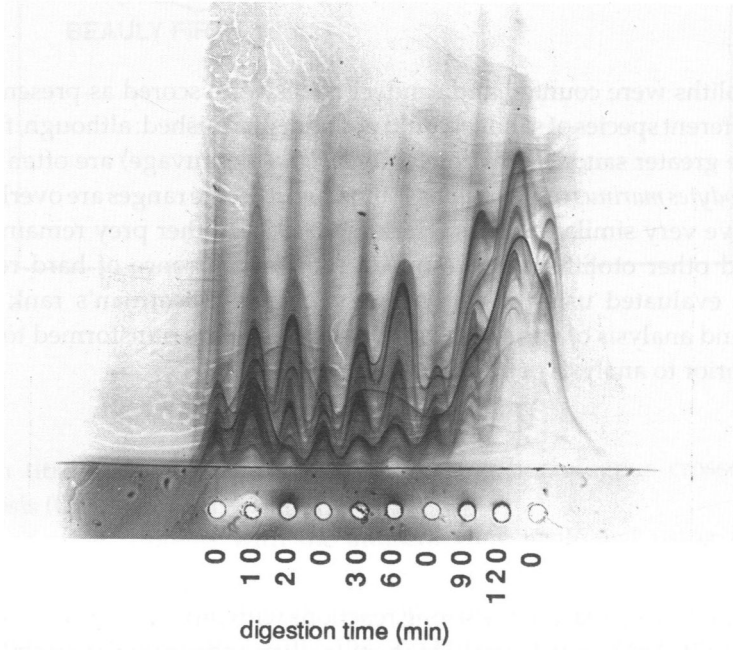

Figure 3. Reaction of sandeel antiserum L with *in vitro* digested sandeel proteins visualised by FRIE. In FRIE the different antigen peaks within a sample are separated on the vertical axis, as a series of 'rockets'. Fusion (linkage) of peaks from adjacent rockets indicates that the same antigen occurs in the linked samples. Several antigenic components of sandeel protein extract are seen to survive prolonged *in vitro* digestion, although becoming less strongly antigenic, as indicated by the increased height of peaks.

Reactions with proteins from other fish

Proteins from many of the other fish species tested reacted with the antisera, in some cases (*e.g.* plaice) strongly, although always producing fewer peaks than the sandeel extract and never fully linking with sandeel protein peaks (Figure 4). Partial linkage, with both standards, was apparent for mackerel, plaice, pollack, poor cod, Norway pout, Norway haddock, and whiting. No linkage was seen for the remaining species tested (Table 1).

Of the fish prepared as *in vitro* digestates, linkage with both standards was seen for cod. The other species tested reacted more weakly as *in vitro* digestates, with no linkage.

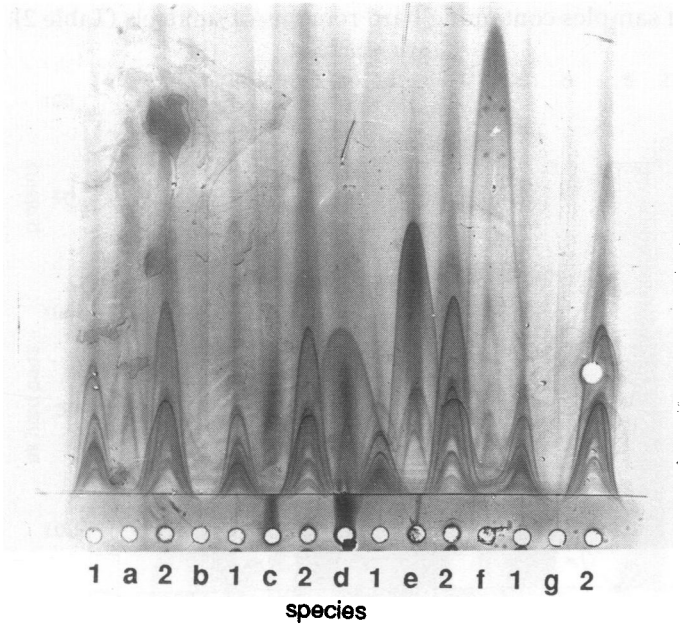

Figure 4. Reaction of sandeel antiserum L with protein extracts from some other North sea fish species (a) megrim, (b) silvery pout, (c) trout, (d) red gurnard, (e) plaice, (f) witch, (g) starry ray. Although there are distinct peaks, *e.g.* for plaice and red gurnard, the peaks do not link with peaks from the standard sandeel extracts 1 (raw sandeel) or 2 (30 min digestate).

Reaction with seal samples

Extracts from all sections of the three digestive tracts containing sandeel remains reacted strongly with the antiserum, with links between sandeel and sample protein peaks. Although the previous results indicate the possibility of linkage occurring with proteins from other fish species, the samples produced numerous peaks, and it is therefore likely that the proteins recognized were from sandeels. Samples from the fourth digestive tract, which contained no sandeel bones, did not react with the antiserum.

Seventeen out of 21 faecal samples from grey seals at Helmsdale reacted with the antiserum, with clear links. Faecal samples from seals fed on diets of cod, herring, and salmon did not react with the antiserum. Some samples from seals fed on mackerel produced a weak reaction, with discernible single peaks, but no links.

Screening of faecal samples from the Moray Firth

The weaker antiserum (K) was used for screening to minimize the likelihood of false positives. Reactions, with linkage, were obtained from 120 out of 248 samples (including the Helmsdale samples; see Figure 5). In most cases where sandeel proteins were recognized, hard parts were also present. However, sandeel proteins were detected in only two thirds of samples containing hard remains of sandeels (Table 2).

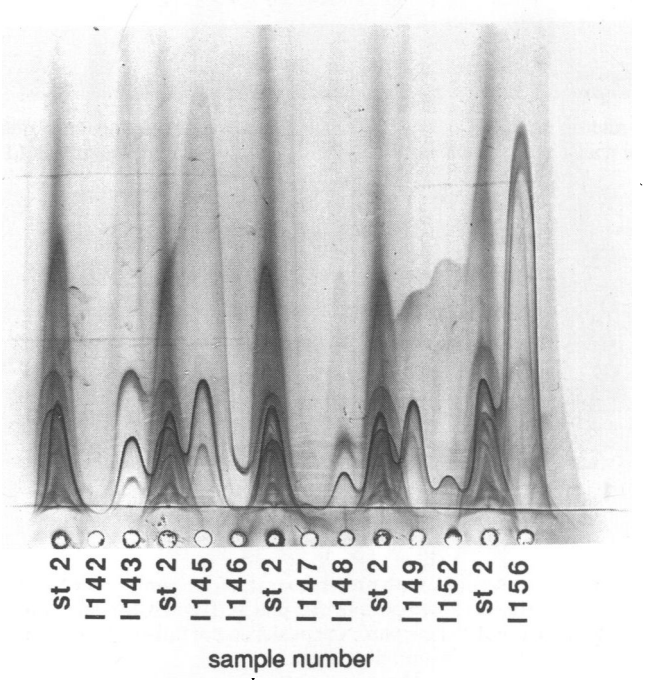

Figure 5. Reaction of sandeel antiserum K with protein extracts from common seal faeces collected in September 1988. Although few recognisable antigens remain, clear linkage is seen between peaks from samples and peaks from standard sandeel extract (st 2).

Table 2. *Frequencies of occurrence for sandeel proteins and hard parts (otoliths or bones) in common seal faeces from the Moray Firth*

		Bones		Otoliths		Hard parts		Total
		-	+	-	+	-	+	
Protein	-	89	39	79	49	72	56	128
	+	9	111	12	108	4	116	120
Total		98	150	91	157	76	172	248

An analysis of variance was performed on log-transformed sandeel otolith numbers, with presence of otoliths from other species, presence of sandeel bones, presence of sandeel proteins, and season (dividing the year into four quarters), as grouping factors. The design was uneven and it was possible to investigate main effects only, ignoring possible interactions between factors. All four grouping factors had a significant effect on the number of sandeel otoliths: presence of otoliths from other fish (F=9·08, P<0·01), presence of sandeel proteins (F=23·4, P<0·001), presence of sandeel bones (F=63·8, P<0·001), and season (F=3·31, P<0·05).

There was a low but significant inverse correlation between the number of sandeel otoliths in a sample and the total number of otoliths from all other species (r=-0·297, N=248, P<0·05).

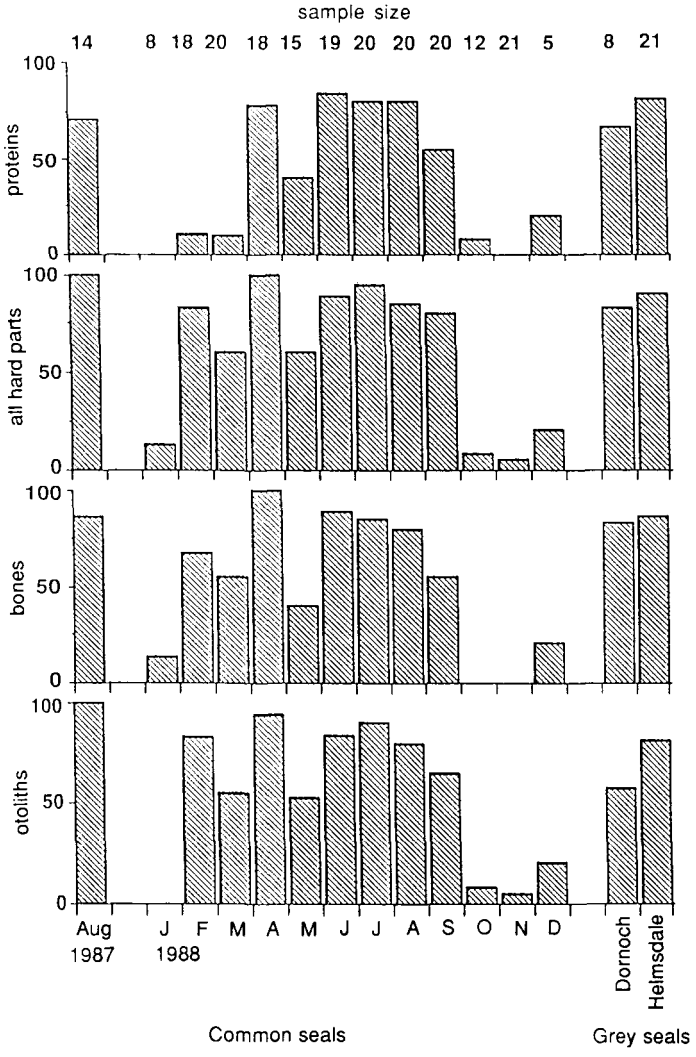

Figure 6. Seasonal variation in percentage frequency of occurrence of sandeels as revealed by different methods of detection.

Samples in which no protein was detected contained fewer sandeel otoliths than samples in which protein was detected (median, M_1=0, N_1=128; M_2=38·5, N_2=120; P<0·001). However, the reverse was true for non-sandeel otoliths (M_1=2·5, N_1=128; M_2=0, N_2=120; P<0·001). Restricting the analysis to samples which contained sandeel bones, the above trends remained for both sandeel otoliths (M_1=6, N_1=39; M_2=49, N_2=111; P<0·001) and non-sandeel otoliths (M_1=0, N_1=39; M_2=0, N_2=111; P<0·01).

Examining the data on a monthly basis (Figure 6), it can be seen that sandeels formed an important component of the diet of seals in the Moray Firth in 1988, particularly in the summer months, when up to 100% of samples contained sandeel remains. The most obvious discrepancy between results from proteins and results from hard parts was in the months of February and March. The number of gadid otoliths (mostly whiting and cod) in the samples was higher for February/March samples (N=39, mean=4·21) than for the rest of the year (N=167, mean=0·52) (Mann-Whitney U test, P<0·001).

DISCUSSION

Tests with proteins from a range of other fish species and with protein residues in seal faeces indicated that the antisera reacted much more strongly with sandeel (*A. marinus*) protein residues than with proteins from other species, and that the likelihood of misidentification was minimal for proteins remaining in seal faeces. However, the extent to which the antisera react with proteins from other species of sandeels has not been evaluated. In contrast to antisera raised to salmon and cod (Pierce *et al.*, 1990b), sandeel antisera reacted strongly with homologous protein residues in seal faeces collected in the field. There is no reason to suppose that sandeel flesh is particularly resistant to digestion, but there are other possible explanations for the difference in antigenicity. In making muscle protein extracts from small fish such as sandeels it was difficult to exclude all other tissues, thus the high residual antigenicity in faeces might derive from, for example, relatively indigestible skin. Another possibility is that smaller prey, *e.g.* sandeels, are less efficiently digested, *e.g.* because they are swallowed whole rather than broken up in the jaws, or pass through the digestive tract relatively quickly.

Sandeel proteins were detected more frequently when larger numbers of sandeels, as determined from otolith counts, had been eaten, suggesting that there is a minimum detectable amount of protein.

The lower rate of detection of sandeels from proteins as compared to hard parts does not imply that the serological approach is less useful. Firstly, the antiserum may not detect all species of sandeel; indeed it is possible that antisera could be used to distinguish the species of sandeel eaten, something which is almost impossible from hard parts. Secondly, proteins may be egested over a shorter timescale than otoliths: there is evidence from captive feeding experiments that some otoliths are retained in seal digestive tracts over several days (*e.g.* Harvey, 1988).

The low rate of detection of sandeel proteins in common seal faeces in February and March 1988, despite the high frequency of occurrence of hard parts, perhaps requires further explanation. Deterioration of proteins during frozen storage is unlikely to be a factor, since detection rates were high in samples from August 1987 which had been

stored over a longer period. It is possible that the seals were feeding on a different species of sandeel, *e.g. A. tobianus* during the first quarter of the year. Changes in seal diet and/or activity patterns may also affect protein detectability due to contingent changes in passage rates (Prime & Hammond, 1987; Harvey, 1988). The number of gadid otoliths present in the samples was high in February and March and it is possible that many of the sandeel remains in these two months were from sandeels eaten by cod and whiting. If so, the serological approach, combined with examination of hard parts, might provide a means to differentiate direct and secondary ingestion.

Both conventional and novel methods indicate that sandeels were an important component of the diets of common seals and grey seals in the Moray Firth, as previously suggested by examination of digestive tract contents (Pierce *et al.*, 1989, in press a). During 1988, there was a clear seasonal pattern in the incidence of sandeels in common seal faeces, with sandeels being particularly prevalent in the summer (see Pierce *et al.*, in press b, for further discussion).

In the present study, antisera were successfully applied to detection of sandeel protein residues in seal faeces samples collected in the field, and the approach is of potentially wide applicability in the study of the role of sandeels, and of other prey species, in marine ecosystems.

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